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The Development of Rumen Microorganisms in Inoculated vs. Isolated Calves[1/]

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This study was made to compare the development of rumen microorganisms in two groups of Jersey calves. The three calves of the isolated group were removed from the dams as soon as they were dropped and were housed in individual pens in a shed in which ruminants had not previously been housed and well away from areas where other ruminants were kept. Equipment and feed for maintenance of the calves were kept in the area and the calves were fed by two persons who had little contact with other ruminants. Overalls, overshoes, and rubber gloves were kept in the area for use when the animals were approached. Most data include only two of the isolated calves as a third animal was found to have a cleft palette and a replacement animal was quite unthrifty and consumed very little feed. The three calves in the inoculated group were housed in the regular calf barn and were inoculated with 100 ml. of fresh rumen contents from a mature cow when they were one, three and six weeks of age.

All calves received colostrum for three days and pasteurized whole milk through 60 days of age. Good quality alfalfa hay (ad libitum) and a grain mixture (15.4% crude protein) were fed after the animals reached ten days of age. Grain was fed to a maximum of 1 lb. until calves were six weeks old and a maximum of 2 lb. thereafter. Rumen content samples for study were collected by stomach tube at 10 to 11 A. M. when the animals were 1, 3, 6, 9, 13, and 17 weeks of age.

Data in table 1 shows that some protozoa were established in inoculated calves at three weeks of age. Two of these calves had the full complement of protozoa at 6 weeks, and the other, at 9 weeks of age. The isolated calves did not show any of the rumen protozoa through 17 weeks of age. When they were inoculated with whole rumen contents, the fauna was quickly established.

Culture counts obtained included a total anaerobic count (rumen fluid-glucose-cellobiose agar), most probable numbers of cellulolytic bacteria (rumen fluid-cellulose liquid medium), total aerobic count (trypticase soy glucose agar), coliforms (violet red bile agar), and lactate fermenters (Gutierrez, J. Bacteriól 66, 123, 1953). The only counts where there appeared to be consistent differences between groups was in the total anaerobic count (table 2) which was

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higher for the isolated group from 6 through 17 weeks of age.

Twenty bacterial colonies were isolated from the anaerobic rumen fluid-glucose-cellobiose agar inoculated from each sample of rumen contents and morphological and certain physiological characteristics were determined. This allowed the presumptive identification of certain groups of bacteria.

A few groups of bacteria common to mature animals were among the predominant bacteria of inoculated calves at 3 weeks of age and many groups were among the predominant bacteria at 13 weeks of age (table 3). Opposed to this, only one group, Selenomonas, was found in isolated calves with any frequency through 17 weeks of age. The fact that one strain of cellulolytic coccus and one of the R-GXCS group were found in the isolated calves indicates that some typical rumen bacteria were present but unable to become predominant. One possible explaination of this observation is that some unknown organisms produce factors necessary for the normal predominance of these bacteria and were not present in the isolated calves.

After the seventeen week samples were taken from the isolated calves, they were inoculated with rumen fluid which was centrifuged to remove protozoa. Two samples from each animal were studied at weekly intervals. Then the animals were inoculated with whole rumen contents and two more samples from each animal were studied. The total anaerobic count was reduced, especially after inoculation with whole rumen contents. The most probable numbers of cellulolytic bacteria were greatly increased after inoculation with protozoa-free rumen fluid and then reduced to the numbers found in the inoculated group after inoculation with whole rumen contents (table 2). Data in table 3 shows that inoculation allowed the bacteria typical of mature cattle, to become predominant in the isolated calves.

These changes in counts and kinds of predominant bacteria after inoculation with rumen contents from a mature animal, as well as the high anaerobic count and failure of normal rumen organisms to become predominant in the isolated calves through 17 weeks of age, indicate that some components of the normal flora are not available to rather strictly isolated calves. A comparison of data on the inoculated calves with data previously collected on calves raised under similar conditions but without inoculation indicates that inoculation does not hasten the development of rumen bacteria in calves raised under normal conditions but does materially hasten the development of rumen protozoa.

Though the number of calves in each group was small, there appeared to be no advantage in weight gains, general appearance, or in TDN consumed per pound of gain in the inoculated group of calves as compared to the strictly isolated group. The two isolated calves made good weight gains and consumed large amounts of roughage. This suggests that other bacteria can satisfactorily carry on the functions of the normal rumen flora.

Table 1 - Direct Microscopic Observations on the Protozoa Present in the Rumen of Growing Calves.

Wks. of Age	KINDS OF PROTOZOA					
	Isolated Calves			Inoculated Calves		
	Entodinia	Diplodinia	Holotrichs	Entodinia	Diplodinia	Holotrichs
1	0/3[1]	0/3	0/3	0/3	0/3	0/3
3	0/3	0/3	0/3	2/3	1/3	0/3
6	0/3	0/3	0/3	3/3	2/3	2/3
9,13,17	0/3	0/3	0/3	3/3	3/3	3/3
20	0/2	0/2	0/2			
24[2]	2/2	2/2	2/2			

1/ Number of calves having protozoa/number of calves observed.
2/ Calves were inoculated with fresh rumen contents from a mature animal 10 days before the 24 week sample.

Table 2 - Culture Counts of Bacteria from Rumen Contents of Isolated and Inoculated Growing Calves.

Age (wk.)	Isolated Calves			Inoculated Calves			
	61	63	Ave.	60	55	64	Ave.
Total anaerobic count, billions per ml.							
1	3.9	4.0	3.9	9.8	7.2	2.1	6.4
3	8.0	12	10	5.5	28	25	20
6	28.0	18	23	0.65	9.5	3.6	4.6
9	17.0	29	23	2.3	4.5	2.5	3.1
13	14.0	12	13	1.2	1.3	1.1	1.2
17	3.0	15	9.0		0.88	0.27	0.57
1/	6.3	11	8.6				
2/	3.8	5.2	4.5				
3/	0.45	0.50	0.48				
4/	0.64	0.36	0.50				
Most probable number cellulolytic bacteria, millions per ml.							
1	<.02	<.02	<.02	16	3.3	<.02	<6.4
3	<.20	<.20	<.20	22	<.20	13	<12
6	7.9	280	144	160	54	540	250
9	.68	26	13	1.7	24	54	27
13	2.2	54	28	110	35	160	100
17	17.0	540	280		92	92	92
1/	70.0	1,800+	940+				
2/	920	1,800+	1,400+				
3/	70	70	70				
4/	160	92	130				

1/ 2/ Calves sampled one and two weeks after inoculation with protozoa-free rumen contents.

3/ 4/ Calves sampled 12 and 19 days after inoculation with whole rumen contents.

Table 3 - The occurrence of predominant bacteria typical of mature cattle in rumen contents of three inoculated calves and two isolated calves

Age (weeks)	Inoculated Calves					Isolated Calves						
	3	6	9	13	17	3	6	9	13	17	1/	2/
Organisms												
(1) Selenomonas	6		1	9	2	5	2	1	2	3		3
(2) Butyrivibrio		5		5	8						11	2
(3) R-GXCS	1	13		4	6				1		12	6
(4) Cellulolytic cocci	3	4	4	1	3		1				8	14
(5) B. succinogenes		2		4	2							2
(6) Others 3/				8	5						1	11
% of total isolates	17	40	8.3	52	43	13	7.5	2.5	7.5	7.5	40	48

1/ Two samples from each animal after inoculation with protozoa-free rumen contents.
2/ Two samples from each animal after inoculation with whole rumen contents.
3/ These include Lachnospira, Succinivibrio, and the ±CR-GXC group.